# 19. Electrophoresis Technology

## Experiment MA-011

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### ABSTRACT

Experiment MA-011, Electrophoresis Technology, was designed to test electrophoresis hardware that would continue the development of technology for electrophoretic separation of materials in the near-zero-g environment of space. Experimental difficulties identified during the Apollo 14 and Apollo 16 electrophoresis demonstrations, if not successfully eliminated, would have severely limited any future separations by electrophoresis of biological materials in space.

The experimental hardware generally functioned as planned. Frozen live cells were successfully transported into space, electrophoretic processing was performed, and viable cells were returned to Earth. A separation of the three types of fixed red blood cells (rabbit, human, and horse) was demonstrated. The human lymphocytes, however, showed no apparent migration. Problems relating to this experiment will be discussed in detail in this report.

The separation of human kidney cells produced the most exciting data. Analysis shows electrophoretic separation throughout the entire column with at least four bands of viable cells. The isotachophoresis experiment definitely demonstrated the isotachophoretic separation of biological cells in a near-zero-g environment.

### INTRODUCTION

The Electrophoresis Technology Experiment demonstrated the concept of static free-fluid electrophoresis in the unique environment of space and verified electrophoresis experiment concepts by operation in this environment. The objectives were as follows:

1. To conduct engineering and operational tests of a space-rated static electrophoresis separation apparatus
2. To further current research efforts through separation of similar cellular species
3. To reduce or eliminate electro-osmosis through the application of a low zeta potential coating to the inner surfaces of the columns
4. To apply biological samples precisely without perturbing the subsequent electrophoresis
5. To maintain viable biological samples during all phases of the experimental procedure
6. To demonstrate isotachophoresis of red blood cells in space

The experiment equipment consisted of four major elements: (1) an electrophoresis unit (EU), (2) a cryogenic freezer (CF), (3) eight experiment columns (six electrophoresis, two isotachophoresis), and (4) eight sample insertion slides.

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The following biological materials were contained in the sample slides for the eight experiment stages.

1. Columns 1 and 5, three species of fixed red blood cells: rabbit, human, and horse
2. Columns 2 and 6, human peripheral blood lymphocytes
3. Columns 3 and 7, human fetal kidney cells
4. Column 4 (isotachophoresis), fixed rabbit and human red blood cells; column 8, (isotachophoresis), fresh rabbit and human red blood cells

To perform the experiment, a crewmember removed an electrophoresis or isotachophoresis column from its storage location and installed it in the EU. Fluid couplings were secured to each electrode chamber of the electrophoresis columns only. The slide containing a specific frozen sample was next removed from the cryogenic freezer and inserted into the column. A camera mounted on the EU cover photographed critical control positions and digital readouts during each column operation. Following each electrophoresis separation, the electrophoresis columns were frozen by the thermoelectric (TE) module and then removed from the cradle. The crewman then quickly removed each electrode chamber from the frozen columns and placed the column in the CF for return to Earth. The isotachophoresis columns were neither frozen nor returned but only photographed in orbit during their operation.

## BACKGROUND

### Electrophoresis

Most biological materials, when dissolved or suspended in a selected aqueous medium, have a characteristic electric charge, and the migration velocity per unit electric field strength (defined as the electrophoretic mobility of the material) is thus fixed (ref. 19-1). If the mobility difference between biological species is small, separation can be enhanced by increasing the length of the column. Electrophoresis can be conducted in liquid media, or free solutions, but problems arise because of disturbances in the bulk of the fluid. Two major causes of these disturbances are sedimentation of the particles or solute being separated and thermal convection generated by Joule heating of the column during electrophoresis. Although various techniques have been developed to overcome these problems on Earth, the elimination of gravity-induced sedimentation and thermal convection can be accomplished best in the near-zero-g environment of space.

Electrophoresis in a free fluid was first demonstrated in space during the Apollo 14 mission. Three materials of different molecular weight, deoxyribonucleic acid (DNA), hemoglobin, and soluble dyes, were to be photographed periodically during electrophoresis in 10-cm (4 in.) long by 0.64-cm (0.25 in.) diameter cylindrical columns. Before operation of the apparatus, the sample materials were isolated in small cavities in a plastic slide that was arranged for insertion in a slot intersecting the separation columns. The experiment was initiated by pushing the slide so that the sample cavities coincided with the separation columns. The diameter of the sample cavity was made slightly smaller than the column diameter so that electrophoresis of the sample would occur away from the walls, where electro-osmotic streaming would be less intense.

Particular problems encountered during the demonstration included a misalined sample insertion assembly that injected the samples near the column walls, poor photography from a handheld camera, and bacterial degradation of the DNA and hemoglobin because of the long storage time. However, a separation was measured between two dyes, and operation of the fluid and electrical systems was normal. The overall results indicated the validity of fluid electrophoresis investigation; therefore, plans were made and apparatus was developed for a second demonstration during the Apollo 16 mission.

The Apollo 16 electrophoresis demonstration was performed using the basic operating elements of the Apollo 14 demonstration, but a nonbiological sample material, polystyrene latex, was used (ref. 19-2). These stable, nondegradable particles were used as models for living cells. Two sizes of latex particles were run separately and collectively to provide comparative data (ref. 19-3). An automatically operated camera, mounted rigidly on the apparatus, photographed a clock, a thermometer,

a column current meter, and the position of the migrating materials. These photographs provided better technical data on electrophoresis in space than had been obtained previously. The sample insertion mechanism was redesigned to provide more reliable release of the samples in the columns. Although each sample was inserted correctly, the insertion mechanism allowed buffer to leak out of the columns before the electrophoresis began, and a bubble formed in each column. The sample bands were severely distorted by electroosmosis, which caused buffer flow along the walls of the columns counter to the direction of electrophoretic migration of the latex particles. Although the parabolic bands observed in space were explained by subsequent analysis, a clear separation of the two sizes of polystyrene latex particles was not obtained because of the electroosmosis. Electrophoresis according to size did occur in the columns containing single species of the latex, and the photographs clearly show distinct boundaries and sharply defined fronts. The deleterious effects of gravity-induced sedimentation and thermal convection on particle electrophoresis can be seen by comparing the results of the Apollo 16 electrophoresis demonstration with those of ground experiments.

For the Apollo-Soyuz Test Project (ASTP), experiment plans included reuse of the parts of the Apollo 14 and Apollo 16 systems that operated correctly, improvement of the techniques used to accomplish sample insertion, elimination of the charge condition along the column walls, and avoidance of bacterial degradation by using proven sterile techniques. A sample insertion technique was devised to ensure precise injection of the sample into the electrophoresis column without affecting the properties of the sample or disturbing the fluid column. The Apollo 14 method of inserting a thin rectangular vane containing cylindrical sample disks into the column was slightly modified and used. Buffer leakage and bubble formation were avoided by specific design techniques. Each sample disk was kept frozen, and the entire insertion slide was stored in a CF that was kept at liquid-nitrogen (77 K (−196° C)) temperatures.

The advantages of a separate insertion vane containing frozen samples are as follows:

1. The frozen disks can be inserted into the column and allowed to thaw in contact with the buffer while any fluid disturbances induced by the sample insertion are damped.
2. Agglutination and sedimentation of the sample material in storage can be avoided by rate freezing a well-mixed sample on the ground.
3. The freezing preserves the viability of the live biological materials.

### Isotachophoresis

Isotachophoresis is a relatively new technique of electrophoretic separation in which a discontinuous electrolyte system is used at the site of sample injection (ref. 19-4). The boundaries between species of different mobility are sharply defined and stabilized by electrical forces. Adjacent compartments of sample components that may not be distinguishable by zone electrophoresis may be resolved by isotachophoresis. The boundaries are highly self-recuperative and will reform if stirred or disrupted by other factors, including convection. The concentration of each substance within a compartment is uniform and remains constant throughout the run once the separation has been achieved. Because of this uniformity, higher concentrations of components can be handled with no deterioration of the sharp boundaries.

## EXPERIMENT SAMPLE SELECTION

The samples selected for the Electrophoresis Technology Experiment were fixed red blood cells, human lymphocytes, human kidney cells, and fresh human and rabbit red blood cells. Eight columns were processed during the experiment; the columns and samples are shown in table 19-I.

### Red Blood Cells

To assess the resolution and sharpness of the bands formed by electrophoresis in space, a well-characterized particle species is required.

*TABLE 19-I.—Columns and Samples Processed in Space*

| *Type of electrophoresis* | *Column no.* | *Sample* |
|---|---|---|
| Zonal | 1 and 5 | Fixed[a] rabbit, human, and horse red blood cells |
| | 2 and 6 | Human lymphocytes |
| | 3 and 7 | Human kidney cells |
| Isotachophoresis | 4 | Fresh rabbit and human red blood cells |
| | 8 | Fixed[a] rabbit and human red blood cells |

[a]Formaldehyde fixed.

Although polystyrene latexes were the model particles for the Apollo 16 demonstration, the red blood cells used on the ASTP flight (erythrocytes fixed in a formaldehyde fixative) provide an almost indestructible sample material resistant to mechanical stress, hemolytic agents, and surface modification. These fixed red blood cells are stable for months under varying temperature conditions, and extensive electrophoresis mobility measurements of the cells using a variety of buffers have been published (ref. 19-5). Each type of cell was selected to be morphologically distinguishable under microscopic examination. The red color of the cells makes them clearly distinguishable on the photographs made in orbit. The isotachophoresis experiment used fresh red blood cells.

## Lymphocytes

The interest in specific lymphocytes stems from the increased emphasis in the field of immunology. Initial interest in immunology grew out of the knowledge that persons who survive an infectious disease seldom contract that disease again. The two major types of immune mechanisms are humoral and cellular. Humoral immunity is characterized by the circulating antibodies that originate from B lymphocytes. Cellular immunity is mediated by means of T lymphocytes and is responsible for delayed allergic and transplant-rejection reactions. With the increasing emphasis on cell interactions and the cellular basis of immunology, a simple method of obtaining pure fractions of cells from heterogeneous populations is desirable. From examination of microelectrophoretic data, differences in cell surface charge are known to exist (ref. 19-6), and differences in electrophoretic mobility have been measured. The significance of the cell separation is of great practical and theoretical importance. Many unresolved questions in immunology depend on the availability of pure lymphocyte subpopulations; such subpopulations are needed to study the nature of the interaction between T and B cells in antibody formation and the nature of the recently described interaction between the classes of T cells in reactions of cell-mediated immunity. More specifically, interest has developed in the clinical use of various soluble lymphocyte products (lymphokines) in a manner analogous to the use of antisera. Several other lymphocyte products, such as the factors modifying inflammatory cell behavior, are also important. The fractionation of these different lymphokines depends on the earlier separation of the specific lymphocyte populations responsible for their production. To date, none have been fractionated to any significant extent.

## Kidney Cells

The isolation and production of the enzyme urokinase (UK) has interested biomedical

laboratories for more than 20 years (ref. 19-7). This enzyme is capable of effecting the conversion of plasminogen to plasmin. This conversion is necessary to accomplish blood clot lysis. Originally, UK was extracted from urine; however, at least 1500 liters of urine were required to produce a single UK unit dose consisting of 4 million CTA (Committee on Thrombolytic Agents) units. The production cost was $1200/CTA unit dose. Another source was subsequently discovered; UK has been isolated from cultures of cells located in the cortex of the kidney as follows. Human fetal kidneys are trypsinized to disperse the cells and then tested for viability. The cells are grown out in cultures on mass tissue propagators from which the UK is harvested. Currently, the demand for UK greatly exceeds the capability to produce it. Bernik and Kwaan (ref. 19-8) in 1969 observed that only approximately 5 percent of the cells in the cortex of the kidney produce UK. Obviously, if these "producing cells" could be isolated and subjected to subculturing techniques, a twentyfold increase in the yield and a corresponding reduction in the cost of this lifesaving drug might result. Electrophoresis has been used to try to isolate these "producing cells." The disadvantages of separation on Earth are the loss of resolving power due to sedimentation of the cells and convective mixing due to the heating effects of electrophoresis. Under zero-g conditions, these disadvantages should be eliminated or minimized.

As outlined, the kidney cell portion of Experiment MA-011 consisted of taking to orbit a mixture of frozen human kidney cells, separating them by electrophoresis, refreezing the resulting bands, and returning them to Earth. They were then grown out in tissue culture and assessed for UK production. In addition, extensive analyses were conducted to characterize the separated cells. The information from these analyses, as well as the data from the tissue culturing, has contributed extensively to the understanding of this very important source of UK.

## EQUIPMENT

The experiment equipment consisted of four major elements: (1) an EU, (2) a CF, (3) eight experiment columns (six for electrophoresis and two for isotachophoresis), and (4) eight sample slides containing the experiment specimens. In addition to this experiment hardware, performance of the experiment required the following items of support equipment: (1) a 70-mm electric camera, (2) an 80-mm lens and extender tube, (3) an intervalometer, and (4) an EU power cable. As stated, the materials used as samples in the experiment consisted of fixed red blood cells of three different types (rabbit, human, and horse), lymphocytes, and human kidney cells. In addition to the slides processed, several control slides containing samples of the biological materials were stored in the CF at launch and remained there throughout the ASTP mission to allow assessment of the effects of space flight on the biological materials.

A general description of experiment operation follows. An electrophoresis or isotachophoresis column was removed from its storage location and installed on the EU column cradle. Fluid couplings were secured to each electrode chamber of the electrophoresis columns only. Then, the proper sample slide with a frozen sample was removed from the CF and inserted into the column. The 70-mm camera, mounted on the EU cover, photographed the experiment column during a run. After each electrophoretic separation was completed, the column was frozen in place on

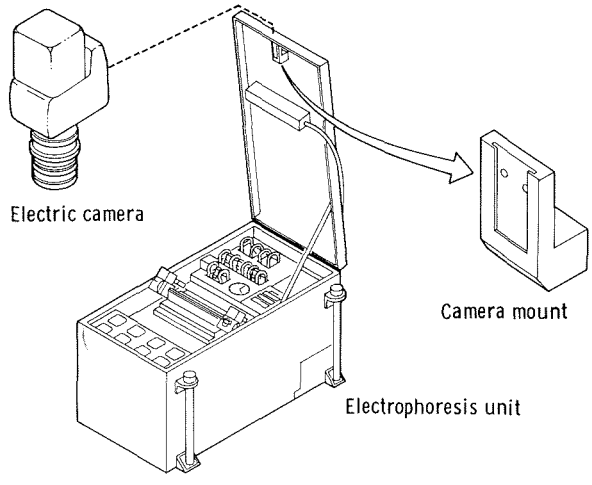



FIGURE 19-1.—Electrophoresis unit with camera.

the EU by the TE module. The frozen column was removed from the column cradle, the electrode chambers were removed and discarded, and the column was placed in the CF for return to Earth. Isotachophoresis specimens were neither frozen nor returned but only photographed. Duplicate electrophoretic separations were performed using each of the three sample materials. The two isotachophoretic separations (runs 4 and 8) were performed using rabbit and human red blood cells. Run number 4 contained formalin-fixed red blood cells and run number 8, fresh red blood cells.

A detailed description of each major hardware element and its subelements is provided in the following paragraphs.

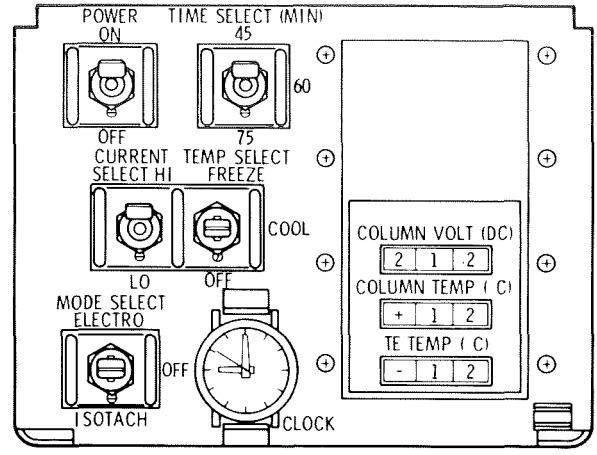



FIGURE 19-2.—Electrophoresis unit control and display panel.

## Electrophoresis Unit

The EU (fig. 19-1) was the apparatus in which the experiment was performed. External envelope dimensions of the unit (cover closed), excluding mounting lugs and fasteners, were 20.32 by 20.32 by 40.64 cm (8 by 8 by 16 in.). Launch weight was 13.61 kg (30 lb). The EU was designed to be mounted on the Electrophoresis Experiment (MA-014) adapter rack near command module locker A-6 with four thermally stable fasteners. Subassemblies provided by the EU were as follows.

1. Controls and displays
2. Fluorescent light assembly for illumination of the experiment columns during processing
3. Thermoelectric module for cooling and freezing the electrophoresis columns
4. Self-contained power supplies that required only an external 208-V ac (115-V line-to-neutral), three-phase, 400-Hz connection
5. Buffer solution reservoir
6. Buffer solution circulation pump
7. Phase separator assemblies for removing gases liberated in the buffer solution
8. Electrical circuitry and plumbing required by the experiment
9. Cover with one knurled screw fastener latch to protect the EU when not in use
10. Camera attachment assembly for mounting the camera to the EU cover
11. Individual storage compartments for eight experiment column assemblies

The EU control and display panel (fig. 19-2) is described as follows. In the ON position, the POWER switch energizes the fluorescent light assembly and the EU digital displays and also activates the TE module cooling blower.

The TEMP SELECT switch has positions FREEZE, COOL, and OFF. The FREEZE position is selected to freeze the electrophoresis column after each electrophoresis separation has been accomplished. A thermoelectric temperature controller allows a minimum temperature of approximately 233 K (−40° C). When the COOL position of the switch is selected, the temperature controller will control the TE units to temperatures as high as 278 K (5° C). The switch is placed in this position at the beginning of viable cell electrophoresis separations and remains in COOL until the separation is accomplished. Because performance of the isotachophoresis portion of the experiment required neither cooling nor freezing of the column, the OFF position was used for isotachophoresis.

The MODE SELECT switch has positions ELECTRO, OFF, and ISOTACH. The ELECTRO position is selected to perform the electrophoresis. When the ISOTACH position of the switch is used to perform the isotachophoresis, the buffer pump, which is not needed for this part of the experiment, does not operate.

The TIME SELECT (MIN) switch is used to select a time period of 45, 60, or 75 minutes for experiment operation.

The CURRENT SELECT switch is used to select the level of current flow through the experiment column to either 4.00 mA (HI) or 1.31 mA (LO). The electrophoresis samples were processed with the switch in the HI position, whereas the LO position was used for isotachophoresis.

The COLUMN VOLT (DC) digitally displays the voltage sensed across the experiment column electrodes. The range of the display is from 0 to 999 V in increments of 1 V, with an accuracy of ±2 percent.

The COLUMN TEMP (°C) displays the buffer solution temperature in the electrophoresis column. The range of the display is from –99° to 99° C in increments of 1° C, with an accuracy of ±2 percent.

The TE TEMP (°C) displays the temperature of the thermoelectric module assembly cold plate. The range of this display is from –99° to 99° C in increments of 1° C, with an accuracy of ±2 percent.

The purpose of the EU clock (a commercial panel-mounted wristwatch) is to enable time correlation of the photographs taken during the experiment process. A metric scale is mounted next to the experiment column cradle. The scale is marked from 0 to 130 mm in 1-mm increments to provide distance information in photographing the biological specimens as the specimens move through the column during the experiment. A portable fluorescent light assembly mounted on the EU cover supplies the illumination required both for viewing the electrophoresis and isotachophoresis processes in the experiment columns and for photography. The fluorescent light tube, contained inside a polycarbonate tube for protection, is permanently bonded to its electrical receptacles.

The EU contains a TE assembly consisting of four three-stage thermoelectric coolers to remove heat from the electrophoresis columns during the experiment and to freeze the columns after each separation has been performed. The four coolers are mounted on a heat sink that is cooled by a flow of 0.09 $m^3$/sec (190 $ft^3$/min) free air by a 208-V ac, three-phase blower. The cradle holding the electrophoresis columns during the experiment is attached to the TE assembly cold plate. The cold plate contains a thermistor device to sense the cold plate temperature. A thermal cover joins the column cradle to provide thermal continuity around the periphery of the column. The thermal cover is designed to remain in place for all freezing operations and during electrophoresis of lymphocytes and human kidney cells. The column assemblies can be isolated from the TE cradle by using special thermal spacers or slide devices.

A buffer solution reservoir is mounted on a removable tray that forms a part of the EU structure. The tray is attached to the EU by screw fasteners. The reservoir contains a cylindrical rubber diaphragm with a hemispherical end and has a buffer solution capacity of 57.7 ml. The buffer solution enters the reservoir from a phase separator assembly and is then delivered from the reservoir to the buffer solution pump. The reservoir was covered with a hydrophobic felt cover to ensure containment of the buffer solution if the diaphragm ruptured.

A double peristaltic pump, mounted on one side of the EU removable tray assembly next to the buffer solution reservoir, circulates the buffer solution through the fluid system to allow removal by the phase separators of gas bubbles

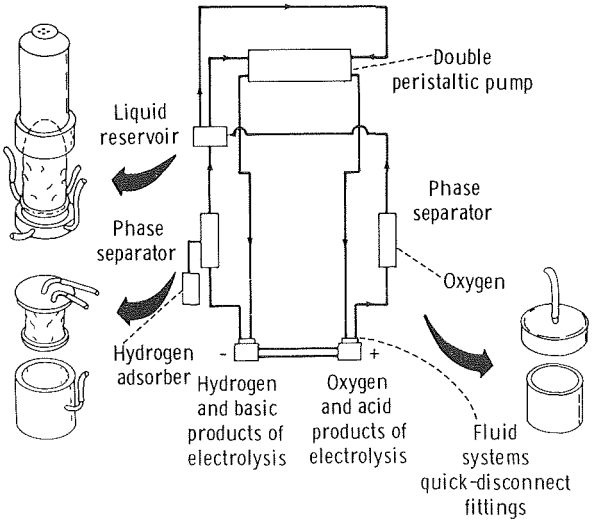



FIGURE 19-3.—Electrophoresis fluid system schematic.

generated at the electrophoresis column electrodes. The pump receives buffer solution from one side of the fluid system (e.g., the column anode side) and circulates it to the other end of the column (fig. 19-3). The capacity of the pump is 35 ml/min.

Two phase separator assemblies are located on the EU removable tray assembly. The phase separators are identical and consist of two cylindrical membranes. One membrane is hydrophilic (porous polyethylene) and the other is hydrophobic (porous polytetrafluoroethylene). The buffer and gas mixture enters between the membranes. The hydrophobic membrane allows passage of gas to either the cabin atmosphere or the hydrogen adsorber, and the hydrophilic membrane allows passage of liquid to the storage reservoir. One separator is connected to the column anode side of the fluid system and removes the oxygen gas liberated in the buffer solution at the anode. The oxygen is vented to cabin air. The other separator is connected to the cathode side of the fluid system and removes hydrogen gas liberated at the cathode. The hydrogen is then routed to the hydrogen adsorber assembly. The separator assemblies return the liquid buffer solution to the reservoir.

The hydrogen adsorber assembly is connected to the phase separator assembly to remove hydrogen bubbles from the buffer solution. The hydrogen gas is adsorbed on a mixture of precipitated palladium (palladium black) and silica gel contained in a fine-filament fiberglass bag in the adsorber canister.

## Cryogenic Freezer

The CF (fig. 19-4) was used to freeze the experiment samples before insertion into the experiment columns for processing and to maintain the frozen electrophoresis columns after the separations had been accomplished. The sample slides were launched in the CF, and the frozen electrophoresis columns were returned to Earth in the CF.

The CF consists of two flasks (one contained within the other). The space between the flasks is evacuated for thermal insulation. The inner flask

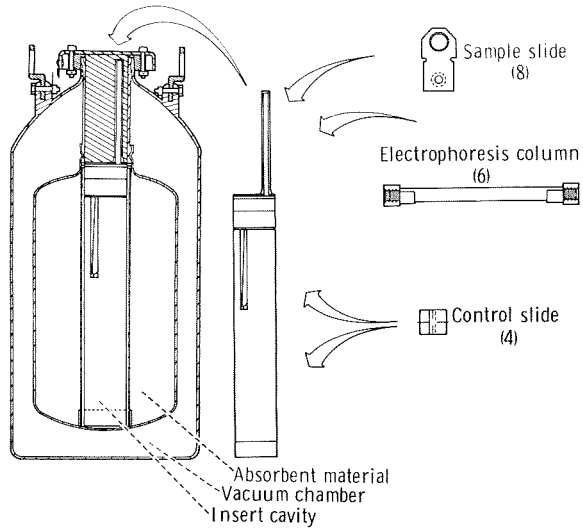



FIGURE 19-4.—Cryogenic freezer.

has an outside diameter of 31.12 cm (12.25 in.) and has a cavity to accommodate a cylindrical sample storage canister with dimensions of 4.98 cm (1.96 in.) diameter and 48.26 cm (19 in.) length. The maximum launch weight of the CF, when charged with liquid nitrogen, is 10.4 kg (22.9 lb).

The cooling medium used in the CF was liquid nitrogen, which was contained in the inner flask and surrounded the sample canister cavity. The CF maintained the samples at a temperature no higher than 188 K (−85° C) for 20 days. The sample canister had space for eight experiment sample slides with samples, one experiment sample column for the German Electrophoresis Experiment (MA-014), and three cylindrical cavities for return of the electrophoresis columns (two columns per cavity).

## Electrophoresis Columns

Six electrophoresis columns (fig. 19-5) were stowed in individual compartments at one end of the EU. Each column consisted of a transparent Pyrex glass tube 0.953 cm (0.375 in.) outside diameter by 0.635 cm (0.25 in.) inside diameter. The tube was 15.24 cm (6 in.) long and was split lengthwise and rejoined with a flexible adhesive

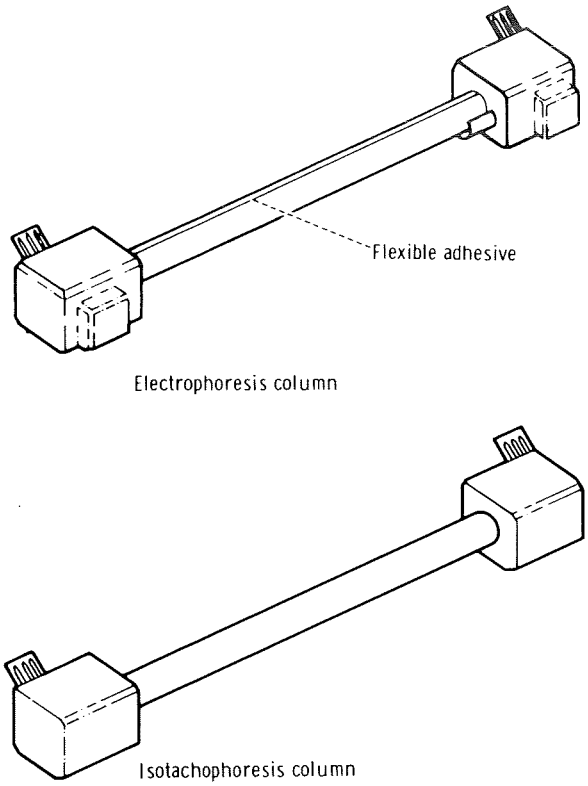



FIGURE 19-5.—Electrophoresis and isotachophoresis column assemblies.

(RTV 140) to allow for expansion of the buffer solution when the electrophoresis columns were frozen. A thermistor bonded to the tube wall at the center of the length of each tube was used to monitor the column temperature for display on the EU COLUMN TEMP (°C) display.

The inside surfaces of the glass columns were stringently cleaned, coated with Z-6040 (γ-glycidoxypropyltrimethoxysilane, Dow Corning) and then coated with methylcellulose in order to obtain a stable, biocompatible surface with close to zero zeta potential. Confirmation of the adequacy of the coating was obtained by experimentally determining the electrophoretic velocity of fixed red blood cells in the coated columns. The ends of the tubes were plugged with a fritted glass disk containing a 3-percent agarose gel. The columns were filled before flight with a sterile buffer solution. Each column was sealed in a polyvinylidene chloride bag that was then placed in a fine-filament fiberglass bag. Attached to the polyvinylidene chloride bag was a wiper used to wipe the female electrical connectors before column installation. Each end of the electrophoresis column has an electrode chamber assembly containing a platinum electrode, a printed circuit electrical connector, and a mating EU buffer solution connector.

The electrode chamber assemblies were removable for disposal after the column had been frozen in the EU. The electrode chamber assembly containing the column cathode had a cavity into which the sample slide was inserted for experiment operation. A blank slide was contained in the cavity before experiment operation. The blank slide had a 0.13-cm (0.05 in.) inside-diameter threaded hole used for preflight filling of the column with buffer solution. The opening was sealed after filling. (The assembly electrical schematic is shown in figure 19-6.)

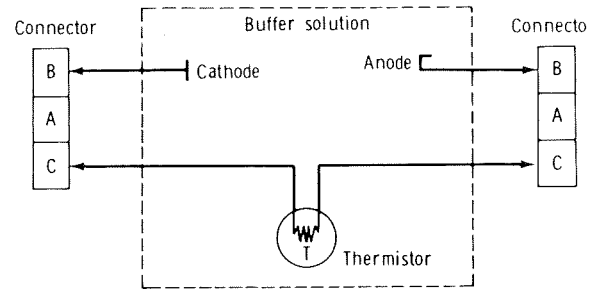



FIGURE 19-6.—Electrophoresis column electrical schematic.

The buffer used in the electrophoresis columns consisted of a mixture of 1.76 mmol $Na_2HPO_4$, 0.367 mmol $KH_2PO_4$, 6.42 mmol NaCl, 0.336 mmol $Na_2EDTA$, 222 mmol glucose, and 514 mmol glycerol in water.[1] It has a hydrogen-ion concentration (pH) of 7.30 ± 0.10 at 293 K (20° C) and a calculated ionic strength of 0.0097 mol/liter. At 298 K (25° C), the conductivity was 0.96 mmho/cm; the density, 1.022; and the dynamic viscosity, 0.00111 N-sec/$m^2$ (0.0111 P).

[1]Na, sodium; H, hydrogen; P, phosphorus; O, oxygen; K, potassium; Cl, chlorine; and EDTA, ethylenediaminetetraacetic acid.

The buffer was demonstrated to be compatible with the column coating, all the components of the EU system, and the candidate materials to be flown on the mission. The mobilities of fixed rabbit, human, and horse red blood cells in this buffer were 1.60, 1.96, and 2.60 $\mu m\ sec^{-1}\ V^{-1}\ cm^{-1}$, respectively.

### Isotachophoresis Columns

Two isotachophoresis columns (fig. 19-5) were stowed in individual compartments in the EU. Each column consisted of a glass tube having dimensions of 0.97 cm (0.38 in.) outside diameter, 0.64 cm (0.25 in.) inside diameter, and 15.24 cm (6 in.) length. Each end of the tube had an assembly containing a flat, disk-shaped, three-lobed electrode (one electrode assembly with a silver anode and the other with a palladium cathode), a rubber diaphragm, and a printed circuit electrical connector.

The assembly containing the palladium cathode had a cavity into which the sample slide was inserted for experiment operation. A blank slide was contained in the cavity before processing the column. The blank slide had two 0.13-cm (0.05 in.) inside-diameter threaded holes used to fill the column with buffer solution before flight.

The isotachophoresis occurred in a column containing a leader buffer of 0.62 ml of 85 percent phosphoric acid in 500 ml water, with 42 g of dextrose and 276 g of glycerol, adjusted to a pH of 7.4 in 1 liter of distilled water. The terminator buffer contained 2 g of serine, 42 g of dextrose, and 276 g of glycerol, adjusted to pH 8.2 in 1 liter of glass distilled water.

### Sample Slides

Each sample to be processed during the experiment was mounted in a slide assembly (fig. 19-7). Before processing, the slides were stowed in the CF sample canister. Each slide assembly was contained in a tetrafluoroethylene cover 0.13 mm (0.005 in.) thick that had a removal tab 5.08 cm (2 in.) long of the same material. The slides were removed from the CF sample canister by grasping the removal tab and pulling the slide out of the canister. Every two slides removed from the canister exposed a return storage space in the canister for two electrophoresis columns. In addition to the slides to be processed, four "control" slides with sample material were stowed in spaces on the circumference of the sample canister. Two control slides contained lymphocytes and two contained kidney cells. These "control" slides were designed to assess the effects of the flight environment on the viable sample materials; they were returned to Earth without processing.

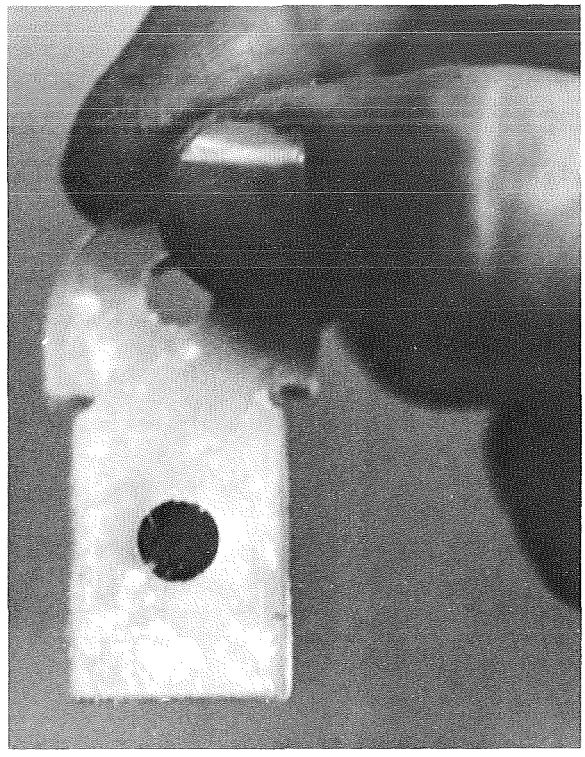


FIGURE 19-7.—Sample slide containing frozen sample.

Each sample slide was numbered to correspond to the appropriate column. The slides were removed from the canister and inserted into the experiment column electrode (cathode) chamber assembly for processing in numerical order. Each slide was discarded together with the electrode chamber assemblies after each electrophoresis column was frozen for return. The entire isotachophoresis column was discarded after processing.

## RESULTS

In the laboratory, the frozen buffer containing the processed samples was separated from the glass electrophoresis columns. These frozen ice columns were sliced into sections for processing by each Co-Investigator. In slicing the columns, several critical requirements had to be satisfied: (1) the frozen column had to be sliced at 233 K (–40° C) or colder; (2) contamination from the slicing mechanism was to be minimized (e.g., heating and particulates from the cutting device were to be avoided); (3) the slices had to be between 0.5 and 5.0 mm long; (4) each slide had to be contained aseptically; and (5) the slicing procedure had to be recorded photographically. After a thorough search of the literature, no existing device was found that could satisfactorily meet all these requirements. Therefore, a device was designed, constructed, and tested for this specific purpose (ref. 19-4). As shown in figure 19-8, the slicing assembly consisted of an extraction assembly in which the frozen flight column was placed. The extraction rod was used to push the ice column into a Teflon tube contained in the slicing assembly. After proper alinement, the razor blade was used to slice "coins" from the frozen column. The 5-mm coins were captured, bagged, and stored at 77 K (–196° C). The entire slicing assembly was operated in a laminar flow hood and in a sterile manner. Subsequent analyses of the cultured kidney cells proved that no detectable contamination resulted from the operation of this device.

The EU, the CF, and the data-collection assembly met the required objectives with satisfactory experimental results. However, the fluid connect lines (located in the electrode housings) of some of the columns were clogged during assembly, and, without a fluid purge of the electrode housing, chemical and gas products of electrolysis accumulated in the electrode region. This accumulation triggered changes in the buffer that were manifested as column voltage fluctuations and pH changes in the column fluid. These anomalies were observed in columns 2, 5, and 6; operations of the other columns appeared normal.

The experimental results are reported according to the order in which the samples were processed. The electrophoresis experiments are presented first, and the isotachophoretic runs are reported in the last subsection.

### Fixed Red Blood Cells

The analyses of the electrophoretic columns containing fixed rabbit, human, and horse red blood cells (columns 1 and 5) consisted of mobility tests and morphological examinations. Both flight photographs and the sliced samples were used.

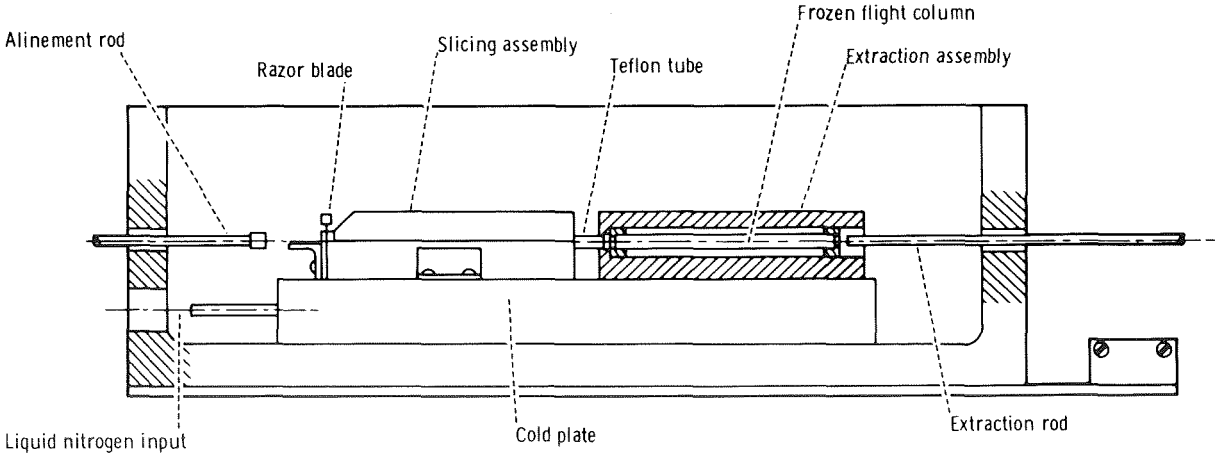



FIGURE 19-8.—Cross section of slicing assembly.

The number of red blood cells loaded in the sample slide was $5.22 \times 10^6$ rabbit cells/0.06 ml, $3.44 \times 10^6$ human cells/0.06 ml, and $7.26 \times 10^6$ horse cells/0.06 ml. The volume of the sample slide for electrophoresis was 0.06 ml.

A major problem in the preparations for the flight experiment was the elimination of electro-osmosis. If present, the fluid flow due to electro-osmosis would cause the cells in the center of the flight column to travel faster than those near the column wall. This effect produces "bullet shaped" bands and reduces the resolution capability of the apparatus. A methyl cellulose coating technique was used to coat the inside walls of the electrophoresis column to eliminate the electro-osmotic flow (ref. 19-9). After use of the coating, electro-osmotic flow was reduced from approximately 4.5 $\mu m\ sec^{-1}\ V^{-1}\ cm^{-1}$ to less than 0.3 $\mu m\ sec^{-1}\ V^{-1}\ cm^{-1}$. With this lower level of electro-osmotic flow (0.3 $\mu m\ sec^{-1}\ V^{-1}\ cm^{-1}$), the bands were predicted to be spread to approximately 2.5 times the length computed for zero electro-osmotic flow after 60 minutes of run time. Not all of this spread would be observable to the naked eye because the concentrations of cells at the boundaries would be lower than the threshold for direct visualization.

In subsequent examinations of the flight photographs, two indications of electro-osmosis were investigated: (1) band-front boundary curvature, which became more pronounced as the run time increased; and (2) longitudinal spread of the bands in excess of that predicted in the absence of electro-osmotic flow.

The band fronts were planar in appearance with no signs of pronounced curvature for column 1. Problems that might interfere with this visual assessment are (1) the optical aberration as a result of viewing bands through a cylindrical tube wall, (2) the haziness of the flight photographs, and (3) the definition of the band boundaries as seen by the naked eye. However, the band fronts appeared planar on visual inspection of the flight photographs.

The spreading behavior of the bands was analyzed by plotting the displacement of the band boundaries from the sample slide as a function of run time. The data were collected by visual examination of 20 flight photographs taken at 3-minute intervals during the 60-minute run of column 1. The bandwidths at 60 minutes were taken from the straight curve fits to the boundary migration data. The results are shown in table 19-II. The bands were slightly more compressed than expected. Thus, it was concluded that no band spreading occurred that was due to electro-osmosis.

The electrophoretic mobility of the particles in the band boundaries was computed from the plot of band boundary migration against run time. The data in table 19-II show that the calculated mobility at 298 K (25° C) for the fast band was 2.36 for the band front and 2.21 $\mu m\ sec^{-1}\ V^{-1}\ cm^{-1}$ for the rear boundary. These values are slightly lower than the predicted average of 2.38 $\mu m\ sec^{-1}\ V^{-1}\ cm^{-1}$ for horse cells as observed in ground-based analytical particle electrophoresis. The front of the slower band complex corresponded to the mean value for human cells of 1.94 $\mu m\ sec^{-1}\ V^{-1}\ cm^{-1}$, but the rear boundary had a mobility of 1.70, which is higher than the 1.56 average value observed for rabbit red blood cells

*TABLE 19-II.—Observed and Predicted Bandwidth Values of the Formalin-Fixed Red Blood Cells*

| *Band* | *Bandwidth, mm* | | *Electrophoretic mobility at 298 K (25° C), $\mu m\ sec^{-1}\ V^{-1}\ cm^{-1}$* | |
|---|---|---|---|---|
| | *Observed* | *Predicted* | *Observed* | *Predicted* |
| Fast (horse) | 8 to 10 | 15 to 18 | 2.21 to 2.36 | 2.38 |
| Slower band complex (human and rabbit) | 20 | 30 | 1.70 to 1.96 | 1.56 to 1.94 |

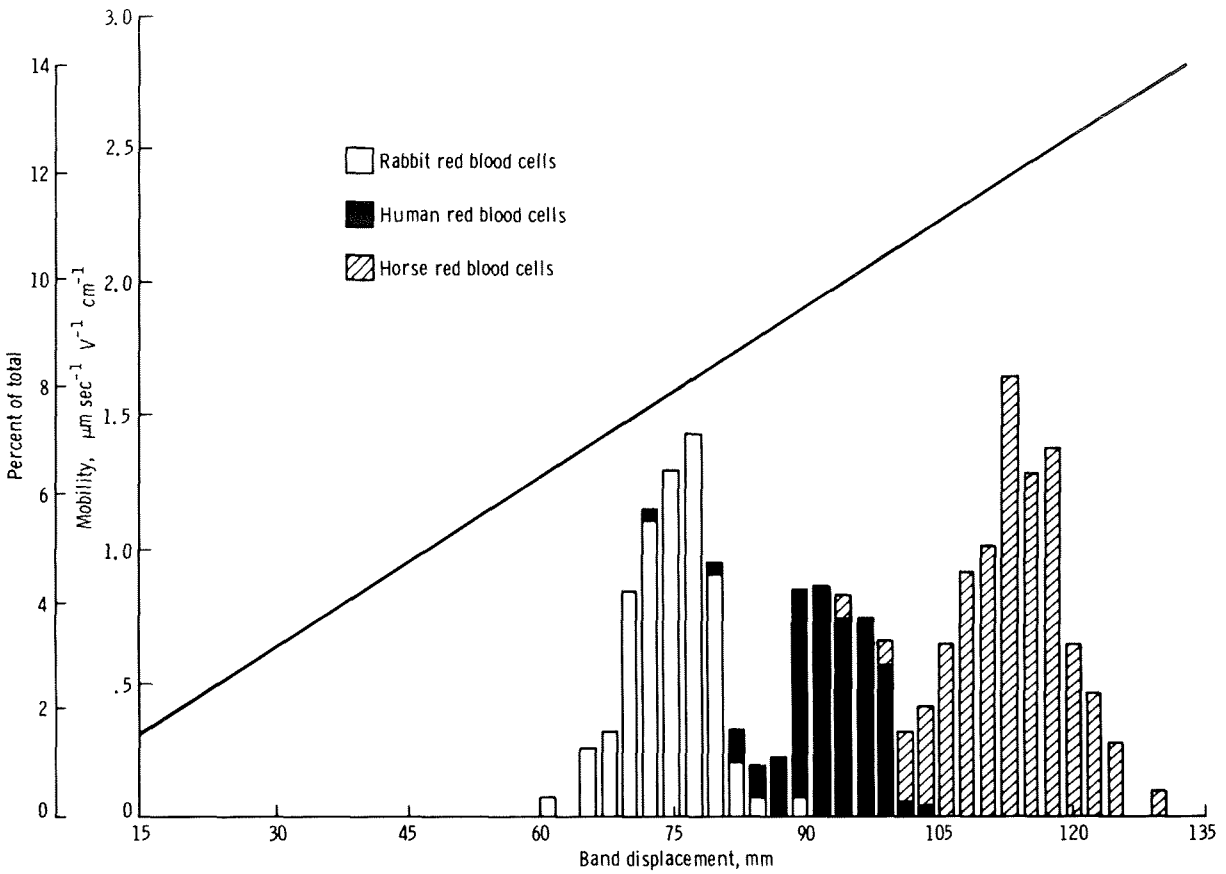



FIGURE 19-9.—Predicted band intensities at various migration distances. Straight line establishes mobilities measured at 298 K (25° C) during ground-based experiments.

in the ground-based experiments. The predicted red blood cell band intensities as a function of migration distance are shown in figure 19-9. This figure was constructed from the observed electrophoretic mobility distributions for each of the red blood cell populations and the number of each cell type loaded in the sample slide. The straight curve establishes the mobilities measured at 298 K (25° C) in A-1 buffer during ground-based experiments. The outside ordinate gives the percentage of the total number of cells in the sample slide found in each bar of the histogram. From figure 19-9, it can be seen that the location of visibly dense bands should be as follows: rabbit, ≈70 to 81 mm; human, ≈88 to 100 mm; and horse, ≈105 to 123 mm.

In figure 19-10, the observed band locations for column 1 at 60 minutes are compared with the

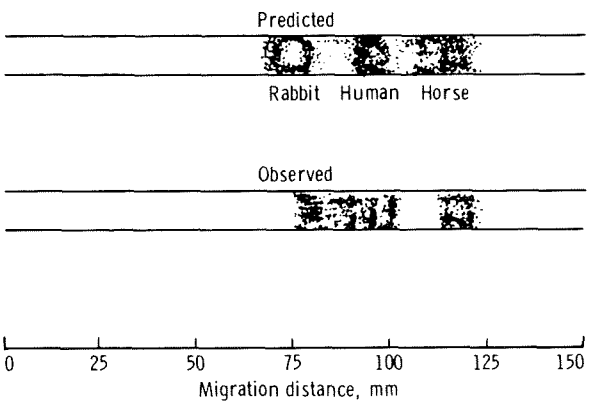



FIGURE 19-10.—Predicted and observed visual migration of formalin-fixed red blood cells. The observed migration of cells is from column 1 of the flight experiment.

predicted locations. The observed band locations were taken from the plot of band migration against run time, because the visualization of the bands was difficult at the end of the run. The fast band corresponded well with the predicted location for horse red blood cells, and the front of the slower band complex corresponded with the predicted human red blood cell location. The rear boundary of the slower band complex was 1 to 1.5 cm farther into the tube than predicted for rabbit cells. Overall, the bands were slightly more compressed than expected, and the separation between horse and human cells was greater than predicted.

The analysis of the flight photographs showed good general agreement between the observed migration behavior of the red blood cell bands and the predicted behavior. Small discrepancies were found and have not yet been explained. Some of these discrepancies may relate to factors not included in the predictive model of the experiment (such as radial and longitudinal thermal gradients in the flight column, compression of the voltage field in the sample disk aperture, and red blood cell concentration effects).

In column 5, two specific instances of sudden disruption of boundaries are indicated in the flight photographs (figs. 19-11 to 19-14). These disper-

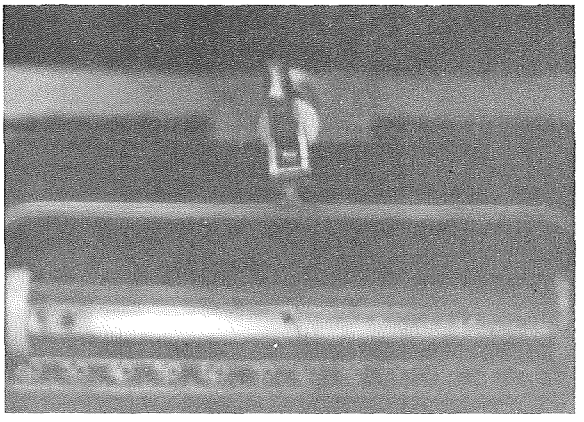


FIGURE 19-11.—Flight photograph of electrophoresis column 5 (AST-26-2182).

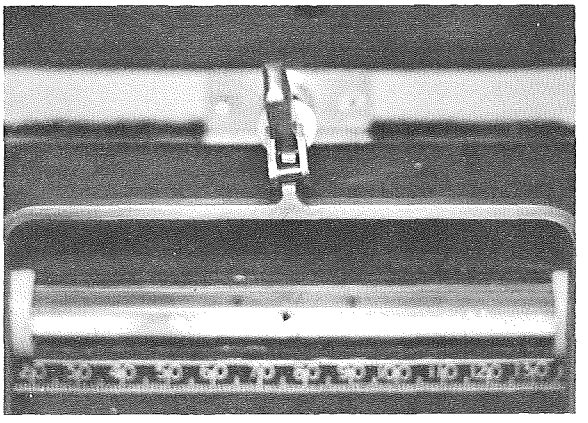


FIGURE 19-13.—Flight photograph of electrophoresis column 5 (AST-26-2186).

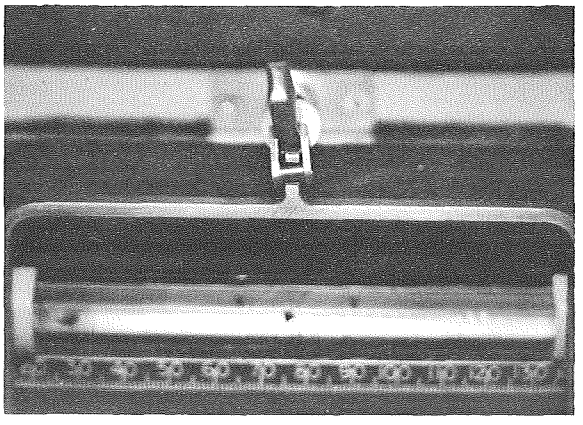


FIGURE 19-12.—Flight photograph of electrophoresis column 5 showing disruption of boundaries seen in figure 19-11 (AST-26-2183).

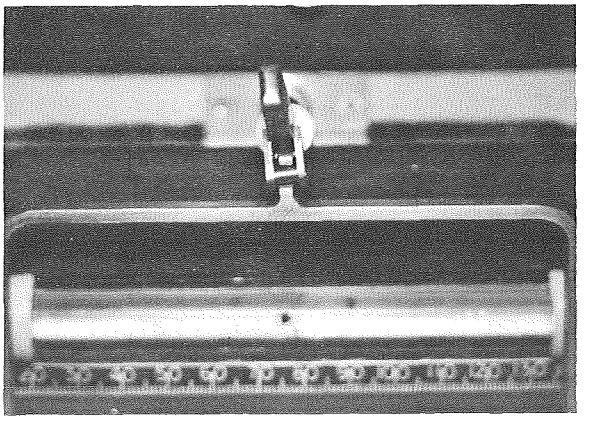


FIGURE 19-14.—Flight photograph of electrophoresis column 5 showing disruption of boundaries seen in figure 19-13 (AST-26-2187).

sions occurred between the time of the photographs shown in figures 19-13 and 19-14. The disruptions probably were caused by random motion of the apparatus or, perhaps, of the spacecraft.

During the sample slicing operation for column 1, the column walls fractured and the column was distorted. The samples were collected in the hope that minimal mixing and contamination had occurred. In the case of column 5, the flight experiment had not functioned properly due to blockage of the electrode buffer circulation ports. This was believed to produce a fluid flow through the electrophoresis column that opposed the direction of particle migration and carried electrolysis products into the column. The sample slicing was performed as planned for column 5.

The calculated recovery of fluid volume was 75 percent for column 1 and 93 percent for column 5. The histograms of cell count compared with the computed distance from the column sample slide are shown for columns 1 and 5 in figure 19-15.

In figure 19-15(a), only six fractions were obtained from the region of the column containing the red blood cell bands. These were placed in the sample histogram starting from the distal end of the column from the sample slide because 25 percent of the column fluid had not been recovered and the earlier sample slices were shown to contain consistently less volume than calculated. The histogram agreed well with the locations of cells in the flight photographs, but this agreement may be fortuitous because cell typing and electrophoretic data indicated considerable mixing of cell types. The recovery of cells was 70 percent of the theoretical value.

In figure 19-15(b), no discrete bands were shown in the histogram for column 5, which is in agreement with the flight photographs. The recovery of cells was 90 percent of the theoretical yield. The front of the continuous band was located at ≈80 mm in the flight photographs in contrast to the location of the cells in the sample slices, where the front was at ≈60 mm. In sample slices 7 to 10, the pH decreased from 7.9 to 6.5 and reached values of 3 to 4 in slices at larger migration distances.

The cells in three sample slices from each column were examined by analytical particle electrophoresis to determine whether they displayed the electrophoretic mobility distributions expected from their locations in the flight column. Data were collected for 100 cells from each sample.

The samples from column 1 slices 1, 3, and 4 all had similar electrophoretic mobilities and the particle analysis is not consistent with flight photographs. Their mean mobilities were all in the range from 2.05 to 2.17, which suggests that the cells had either been mixed, contaminated during the column breakage, or mixed before freezing. The cells in sample slices 5, 8, and 11 from column

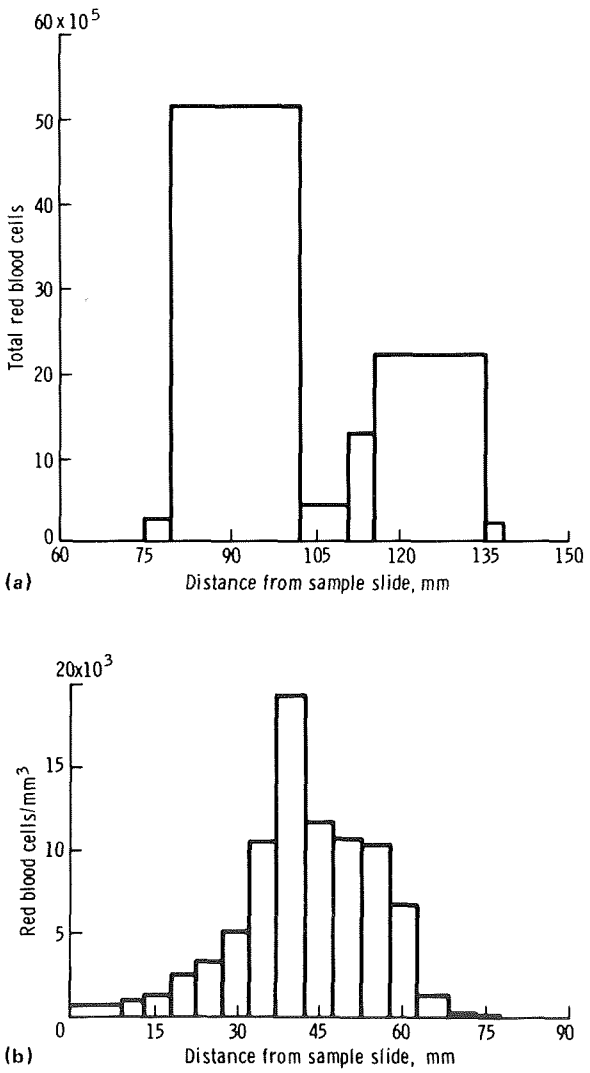
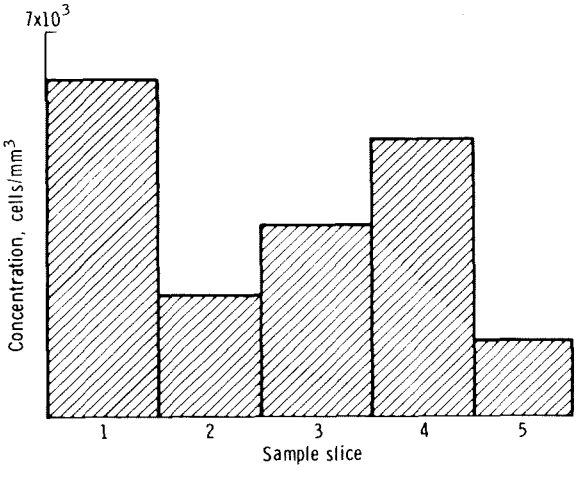



FIGURE 19-15.—Histogram showing cell counts. (a) Column 1. (b) Column 5.

5 showed a trend from lower to higher electrophoretic mobilities, respectively, but the separation was not large.

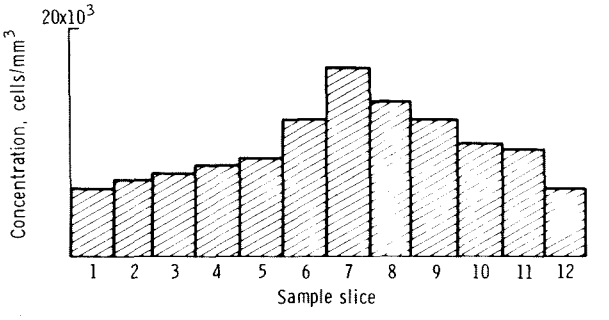
Estimations made of the numbers of each cell type from the electrophoretic mobility data were in general agreement with the morphological typing estimations (figs. 19-16 and 19-17). For column 1, the typing data showed similar distributions of the three cell types in each of the sample slices in contrast to the expected behavior. This finding supported the suggestion that the samples had been mixed either when the column broke or before freezing. As expected, the data for column 5 show that there were enrichments of rabbit cells in the slow region of the band (left end of column) and horse cells in the fast region (right end of column).

The results of the postflight sample slice analyses indicate that the samples from column 1 are of little use in interpreting the behavior of the flight experiment. The column 5 data show partial separation of cells according to their electrophoretic mobilities. Electro-osmosis, which is a major obstacle to successful electrophoretic separations in a closed cylindrical tube apparatus, was eliminated in the ASTP electrophoresis experiment. The coating techniques developed for this purpose significantly increased the resolution capability of the electrophoresis unit and will be useful for ground-based and future space electrophoretic applications. The red blood cell bands were planar and showed no signs of longitudinal spreading. The MA-011 electrophoresis unit has demonstrated the capability of separating small volumes of sample cells on the basis of their electrophoretic mobilities.




| Sample slice | Cell type | | |
|---|---|---|---|
| | Rabbit | Human | Horse |
| Mobility, percent | | | |
| 1 | 19 | 22 | 59 |
| 3 | 11 | 17 | 72 |
| 4 | 12 | 38 | 50 |
| Morphology per 100 cells | | | |
| 1 | 17 | 26 | 57 |
| 3 | 19 | 48 | 33 |
| 4 | 15 | 31 | 54 |

FIGURE 19-16.—Mobility and morphological data from column 1.




| Sample slice | Cell type | | |
|---|---|---|---|
| | Rabbit | Human | Horse |
| Mobility, percent | | | |
| 5 | 18 | 60 | 22 |
| 8 | 24 | 48 | 28 |
| 11 | 23 | 42 | 35 |
| Morphology per 100 cells | | | |
| 1 | 56 | 38 | 6 |
| 2 | 66 | 31 | 3 |
| 3 | 60 | 40 | 0 |
| 4 | 64 | 36 | 0 |
| 5 | 36 | 57 | 7 |
| 6 | 47 | 41 | 12 |
| 7 | 31 | 32 | 37 |
| 8 | 17 | 41 | 41 |
| 9 | 12 | 27 | 61 |
| 10 | 11 | 23 | 66 |
| 11 | 10 | 34 | 56 |

FIGURE 19-17.—Mobility and morphological data from column 5.

### Human Lymphocytes

Approximately $1.5 \times 10^7$ human lymphocyte cells were loaded into the 0.06-ml volume of each sample slide. Migration of the cells was not detected in either column. In column 2, current was established for less than 3 minutes because of fluid line blockage, which resulted in the formation of gaseous products around the electrodes. These gas bubbles blocked the current and prevented operation of the experiment. In addition, the cell viability was only 6 percent (table 19-III). No explanation can be found for this observation; the pH of the column fluid varied from 6.69 to 6.85, which should not kill the cells.

In column 6, current existed for approximately 30 minutes. In this case, the electrode housings were returned and an investigation confirmed that the right electrode chamber fluid lines were blocked. This condition resulted in an acid pH throughout the column. The pH of the fluid in column 6 was 2.54 on the left end, 2.28 in the center, and 2.21 on the right end. As would be expected, the viability of the lymphocytes was only 1 percent (table 19-III).

In addition, the control cells that were taken into orbit but not thawed showed an unusually low viability (table 19-III). The authors have deduced that, because of the small size of the control slides, these lymphocytes froze and thawed

*TABLE 19-III.—Cell Count and Viability Results of Human Lymphocytes*

| *Fraction no.* | *Count per sample* | *Viability, percent* | *Fraction no.* | *Count per sample* | *Viability, percent* |
|---|---|---|---|---|---|
| | *Column 2* | | | *Column 6* | |
| Left end | 364 000 | 6 | Left end | 392 000 | 0 (clumps) |
| 1 | 44 800 | 6 | 1 | 427 000 | 1 |
| 2 | 50 800 | 0 | 2 | 856 800 | 0 (clumps) |
| 3 | 1 120 | 0 | 3 | 851 200 | 0 |
| 4 | 0 | -- | 4 | 6 160 | 0 |
| 5 | 560 | 0 | 5 | 0 | -- |
| 6 | 0 | -- | 6 | 0 | -- |
| 7 | 0 | -- | 7 | 0 | -- |
| 8 | 0 | -- | 8 | 0 | -- |
| 9 | 0 | -- | 9 | 0 | -- |
| 10 | 0 | -- | 10 | 0 | -- |
| 11 | 0 | -- | 11 | 0 | -- |
| 12 | 0 | -- | 12 | 0 | -- |
| 13 | 0 | -- | 13 | 0 | -- |
| 14 | 0 | -- | 14 | 0 | -- |
| 15 | 0 | -- | 15 | 0 | -- |
| 16 | 0 | -- | 16 | 0 | -- |
| 17 | 0 | -- | 17 | 0 | -- |
| 18 | 0 | -- | 18 | 0 | -- |
| 19 | 0 | -- | 19 | 0 | -- |
| 20 | 0 | -- | 20 | 0 | -- |
| 21 | 0 | -- | 21 | 0 | -- |
| 22 | 0 | -- | 22 | 0 | -- |
| 23 | 0 | -- | 23 | 0 | -- |
| 24 | 0 | -- | 24 | 0 | -- |
| 25 | 0 | -- | Right end | 0 | -- |
| 26 | 0 | -- | Ground control C | 1 290 000 | 19 |
| Right end | 0 | -- | Flight control C | 2 360 000 | 14 |
| Ground control A | 1 420 000 | 46 | | | |
| Flight control A | 3 620 000 | 13 | | | |

faster than the prescribed rate. In preflight tests, the cells routinely showed a recovery of 55- to 63-percent viability when frozen and thawed using the sample keys. These sample keys are much larger than the control slides, although their cell compartments are the same size.

These data are not adequate for conclusions related to the feasibility of space electrophoresis of human lymphocytes. As an application of this work, however, the freezing of human granulocytes, by the same method developed for the freezing of human lymphocytes for ASTP, appears most promising and is being pursued further. The viability of human granulocytes, using the ASTP method, after freezing and thawing is approximately 82 to 86 percent. Also, their phagocytic activity appears undiminished. During simulation of, and in preparation for, the flight experiments, a one-g method for preparative cell electrophoresis was developed that enabled separation of the fastest migrating cells (the T lymphocytes) in a pure state (ref. 19-10).

### Human Kidney Cells

For the human kidney cell experiments, approximately $2.0 \times 10^6$ cells were loaded into the sample slide. Only column 3 was subjected to electrophoresis in orbit; during orbital operations, the crewmembers discontinued the processing of column 7.

The frozen slices obtained from column 3 were thawed rapidly at 310 K (37° C), centrifuged, and resuspended in growth media. These fractions were weighed and the pH determined (table 19-IV). An aliquot was taken so that a viable cell count could be made; and, based on this information, the cells were cultured. The distribution of viable cells is shown in figure 19-18, in which approximately four subpopulations of cells can be identified.

After 28 days, only fractions 11 to 19 had reached confluency. The other fractions were removed from the culture plates and tested for UK activity by the fibrin plate method (ref. 19-11). These fractions showed no fibrinolytic activity. The disposition of the confluent plates and of the subcultures is shown in table 19-V. The cells that were put on production media were tested at various times for UK activity; the cells that were subcultured were removed from the dishes with EDTA and then recultured.

Table 19-VI shows the results of the UK production obtained with the primary and subculture 1 cells after 35 days on production media. There is an obvious enrichment of UK activity in fraction 15. An increased production is also apparent in several other fractions. A control experiment with the same cells cultured using ground-based conditions resulted in the value of 0.28 UK units per 100 cells. The subculture 2 cells did not produce UK when placed on production media. Some of the cells from subculture 2 were analyzed for electrophoretic mobility distribution, and the results of three such fractions are shown in figure

*TABLE 19-IV.—Weight and pH of Sample Slices From Column 3*

| *Fraction no.* | *Weight, g* | *pH* |
|---|---|---|
| Right | 0.3231 | 7.25 |
| Left | .27805 | 7.05 |
| 1 | .15400 | 7.8 |
| 2 | .15110 | 7.9 |
| 3 | .14195 | 7.8 |
| 4 | .16695 | 7.8 |
| 5 | .16320 | 7.85 |
| 6 | .15825 | 7.6 |
| 7 | .14845 | 7.85 |
| 8 | .16250 | 7.75 |
| 9 | .17195 | 7.75 |
| 10 | .15805 | 7.85 |
| 11 | .15435 | -- |
| 12 | .17765 | 7.65 |
| 13 | .15620 | 7.55 |
| 14 | .10100 | 7.45 |
| 15 | .13340 | 7.5 |
| 16 | .13925 | 7.65 |
| 17 | .20405 | 7.25 |
| 18 | .14975 | -- |
| 19 | .12015 | 7.45 |
| 20 | .14915 | -- |
| 21 | .16115 | -- |
| 22 | .16695 | 7.45 |
| 23 | .16230 | -- |
| 24 | .15635 | -- |
| 25 | .18545 | -- |
| 26 | .11970 | 7.4 |

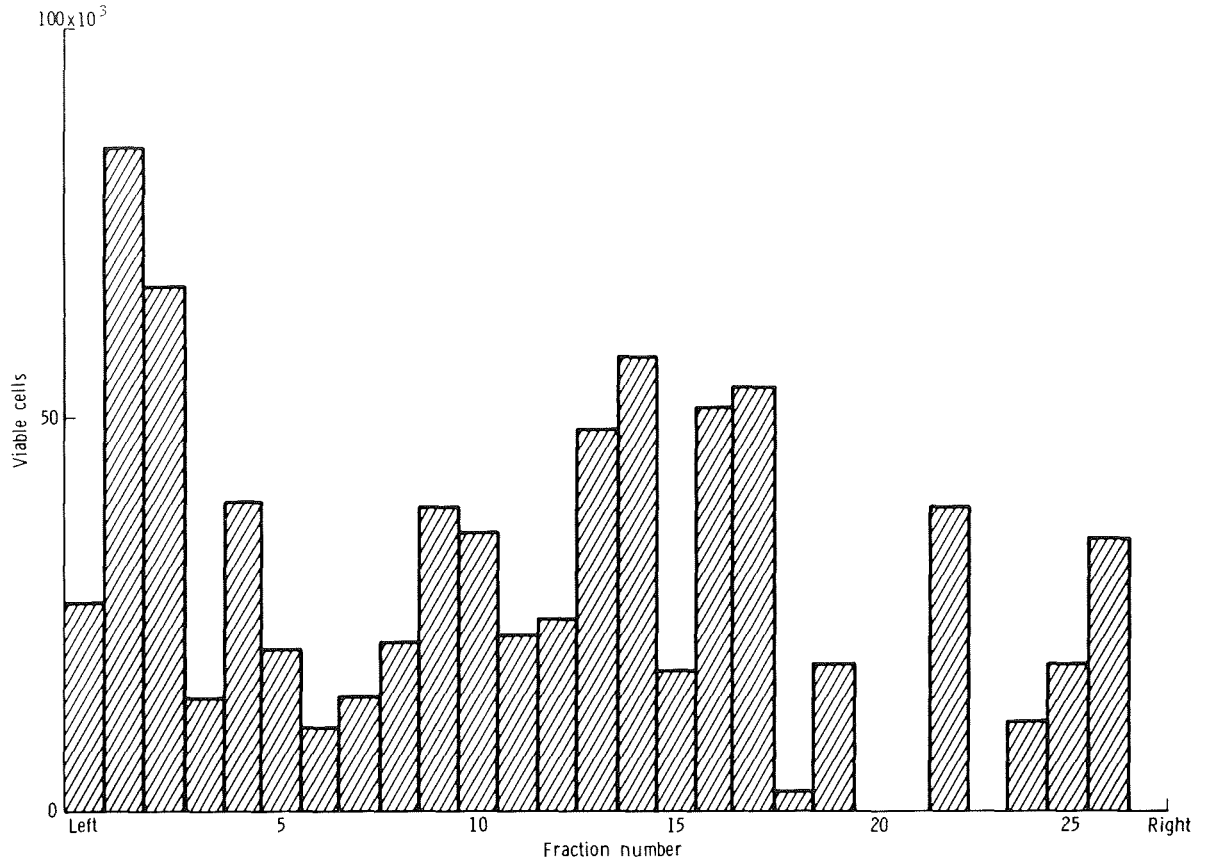



FIGURE 19-18.—Histogram of viable kidney cells for each sample slice in column 3.

19-19. The cells of subculture 1 were also tested for the presence of human granulocyte conditioning factor (HGCF), and the results are shown in table 19-VII. It is evident that the highest concentration of these HGCF-producing cells was in a separate band (fraction 17).

These results show that cells can be separated under sterile conditions and returned from orbit in such a manner that they retain their ability to grow in culture. Electrophoresis in space resulted in good separation of the kidney cells into subpopulations, with an indication of at least three and maybe four subpopulations. This result is in agreement with the best data obtained using the endless belt electrophoresis. Although subpopulations have been observed using the endless belt apparatus, sterile collection of these bands has not been possible. Whereas each fraction from the ASTP flight showed viable cells by the stain technique (ref. 19-12) and they all attached to the glass surface, only the few fractions between 11 and 20 multiplied. The reason for this is not known. The only possible explanation is that the "nongrowers" were more sensitive to unfavorable conditions and therefore could not recover to grow in culture.

During the final quarter of this study, analyses were made for erythropoietin activity. All assays were performed using a standard with a potency of 2.2 units/mg. The harvests from the primary culture, subculture 1, and subculture 2 were assayed for erythropoietin activity. The results, reported in table 19-VIII, are the average of two sets of assays. The results are most encouraging because the peak activity for erythropoietin is found in fraction number 14. This is a different

*TABLE 19-V.—Disposition of Cultures From Column 3*

| *Fraction no.* | *Condition* | *Disposition* |
|---|---|---|
| | *Confluent primary kidney cell cultures* | |
| 11 | Confluent | Production |
| 13 | Confluent | Production |
| 14 | Confluent | Subculture[a] |
| 15 | Confluent | Production |
| 16 | Confluent | Subculture |
| 17 | Confluent | Production[b]; subculture[b] |
| 19 | Confluent | Subculture |
| Flight control B | Confluent | Production |
| Flight control D | Confluent | Production |
| | *Kidney cell subculture 1* | |
| 14-1 | Confluent | Subculture |
| 14-1 | Confluent | Production |
| 16-1 | 50 percent confluent | Production |
| 16-1 | 50 percent confluent | Reculture[c] |
| 17-1 | Confluent | Subculture |
| 17-1 | Confluent | Production |
| 19-1 | Confluent | Subculture |
| 19-1 | Confluent | Production |
| | *Confluent kidney cell subculture 2* | |
| 14-2 | Confluent | Production |
| 14-2 | Confluent | Mobility[d] |
| 17-2 | Confluent | Production |
| 17-2 | Confluent | Mobility |
| 19-2 | Confluent | Production |
| 19-2 | Confluent | Mobility |

[a]Two 35-mm petri dishes.
[b]One 35-mm petri dish.
[c]No growth resulted from this subculture.
[d]Electrophoretic mobility determination.

fraction than for either UK (fraction 15) or HGCF peak (fraction 17). These data indicate that a different subpopulation of cells is responsible for each activity assayed.

In summary, these data indicate an enrichment of UK-producing cells in the area centering around fraction 15. These results can be interpreted to show an incomplete resolution between a producing cell population and a nonproducing population. Considering that the experimental conditions (buffer run time, etc.) were not optimized for kidney cells but generalized for three different separations, these results are not surprising. This lack of optimization is probably the reason for the failure of the cells to produce farther than subculture 2, when normally under optimized growth conditions the cells produce to subculture 7. It also appears that the bands that produced HGCF and erythropoietin do not coincide with that band of cells that produce UK. This is a very interesting finding and indicates that these products are most likely not produced by the same cell.

The analytical mobility data of the subculture 2 show each fraction at this stage to have a rather broad distribution. This was disappointing

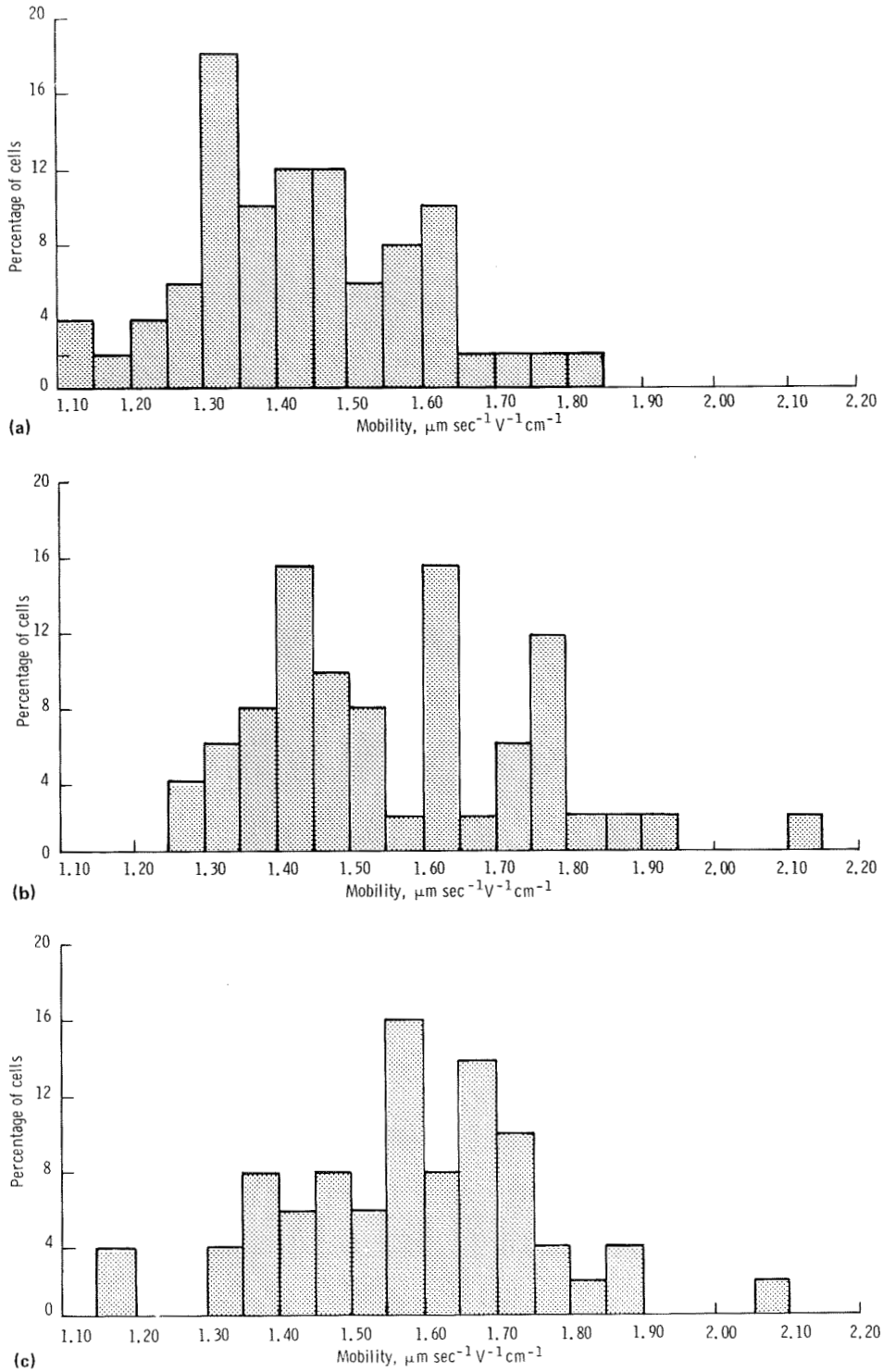



FIGURE 19-19.—Analytical electrophoretic mobility distribution. (a) Cell subculture 2 of fraction number 14. (b) Cell subculture 2 of fraction number 17. (c) Cell subculture 2 of fraction number 19.

*TABLE 19-VI.—Units of UK Produced From Primary and Subculture 1 Kidney Cells Separated in Flight Column 3*

| *Fraction no.* | *UK assay, units/disk* | *Viable cells* | *UK, units/100 cells* |
|---|---|---|---|
| | | *Primary* | |
| 11 | 45 | $0.07\times10^5$ | 0.64 |
| 13 | 535 | .696 | .77 |
| 15 | 240 | .12 | 2.0 |
| 17 | 225 | .744 | .3 |
| B control[a] | 61 | .068 | .9 |
| D control[a] | 81 | .288 | .3 |
| | | *Subculture 1* | |
| 14-1 | 85 | $0.60\times10^5$ | 0.14 |
| 16-1 | 124 | .132 | .94 |
| 17-1 | 205 | .9 | .23 |
| 19-1 | 359 | .222 | 1.62 |

[a]Samples taken as controls on the ASTP mission.

*TABLE 19-VII.—Levels of HGCF Found in Various Fractions of Subculture 1*

| *Fraction no.* | *HGCF, no. of colonies formed*[a] |
|---|---|
| 14-1 | 40 |
| 16-1 | 65 |
| 17-1 | 123 |
| 19-1 | 0 |

[a]Number of colonies formed was corrected for control plate.

because a rather sharp mobility distribution was anticipated. The explanation for this finding may be that the starting fraction is heterogeneous and the cell attachment and growth pattern is a random event that can broaden at each reculture level. It also must be remembered that the mobility data were taken from subculture 2 cells and not from the original cells separated during the ASTP flight. Further analyses should help prove this point and are in progress.

*TABLE 19-VIII.—Units of Erythropoietin Produced From Primary Culture, Subculture 1, and Subculture 2 Kidney Cells Separated in Flight Column 3*

| *Fraction number* | *Erythropoietin activity, units/ml* |
|---|---|
| *Controls*[a] | |
| B | 0.8 |
| D | 0 |
| *Primary* | |
| 13 | (b) |
| 15 | (b) |
| 17 | (b) |
| *Subculture 1* | |
| 14-1 | 1.1 |
| 17-1 | .9 |
| 19-1 | 1.65 |
| *Subculture 2* | |
| 14-2 | 5.0 |
| 17-2 | 2.0 |
| 19-2 | 1.0 |

[a]Samples taken as controls on the ASTP mission.
[b]Inhibitory.

### Isotachophoresis

A disadvantage of isotachophoresis is that the sample compartments are contiguous to each other and never separate to form an intermediate zone of clean buffer. (The buffer in front of the sample is called the "leading buffer" and the buffer that is behind the inserted sample is called the "terminator buffer.") If separation is desired, a so-called "spacer ion" of suitable intermediate mobility must be added. As will be discussed subsequently, this is of crucial importance for isotachophoresis. A further complication is that the heating of the column is not uniform; i.e., the progressively lower mobility compartments are exposed to proportionately higher potential gradients to ensure identical mobility. The temperature is a complicated function of both radial cooling and frontal migration of each compartment. The highest temperature is reached in the terminator buffer. This characteristic caused considerable concern in the design of the ASTP experiment, and it caused both the applied field and the duration of each experiment to be limited to an absolute minimum that was deemed compatible with sample resolution.

The sharpness of isotachophoretic boundaries gives rise to equally steep concentration (i.e., density gradients); thus, gravity effects are even more difficult to overcome than in zone electrophoresis. Because of gravity, protein isotachophoresis has always been conducted only in gels; and, at the beginning of the planning for the ASTP experiment, there was no ground-based apparatus in which cell isotachophoresis could be conducted. Therefore, the ASTP experiment provided a unique opportunity to study cell isotachophoresis.

Approximately 6 months before the ASTP flight, a new apparatus for cell isotachophoresis was developed as a result of extensive ground-based research. The apparatus utilized boundary stabilization through rotation of a horizontal tube, and the apparatus, named Rotofor, permitted the first ground-based work on cell isotachophoresis. Unfortunately, the leadtime for the ASTP experiment preparation was too short to permit as thorough an evaluation as desired.

From the beginning of the planning for the ASTP experiment, the possibility of processing either fixed or fresh red blood cells was considered. Freezing fixed cells presented no problems, but freezing fresh cells was more difficult. Although low-temperature storage of erythrocytes is well documented, the particular requirements of isotachophoresis called for a freezing mixture with low overall electrolytes and substitution with a buffer suitable for isotachophoresis. This latter requirement presented the greatest problem. Many of the usual leader/terminator buffers, although compatible with the freezing of the red cells, caused cell aggregation during an actual isotachophoresis run. Using the Rotofor instrument, nonaggregating buffers were eventually found. In addition, the proposal to use liquid nitrogen as the freezing agent, together with the prescribed method of thawing (direct contact of the frozen cells with buffer at ambient temperature), imposed further limitations on the method of freezing red blood cells. The toxicity of dimethyl sulfoxide, which is widely used in red blood cell preservation, precluded its use in the manned flight experiment. Glycerol, therefore, seemed the most suitable cryoprotectant, especially in view of its electrophoretic inertness.

The primary buffer variables investigated were concentrations of glycerol and dextrose, which were necessary to preserve isotonicity in the freezing mixture. In addition, the influence of variable cooling rates on red blood cell recovery was studied. Good recovery (60 percent) of fresh red blood cells was obtained using a freezing mixture containing 4.2 percent dextrose and 2.8 to 3.5 M glycerol. Since these results were based on a freezing temperature of 194 K (−79° C), the study was extended by using liquid nitrogen for freezing. It was found that, over a wide range of red blood cell concentrations in the freezing mixture (5 to 50 percent hematocrit), no modification in the composition of the cryoprotectant was necessary.

A ground-based problem encountered in isotachophoresis of red blood cells was aggregation. This aggregation was initially absent but became apparent several minutes after application of the current, and it increased progressively during a run. Several leader/terminator buffer combinations were tried, including 2-amino 2-(hydroxymethyl-1, 3-propanediol) hydrochloride, acetate, or cacodylate as leader and

threonine, alanine, or asparagine as terminator; all of these caused aggregation. The combination that prevented aggregation was the use of phosphate as leader and serine as terminator.

Using nonfrozen red blood cells in phosphate serine buffer systems, isotachophoresis separation of cells from hemoglobin (or various added dyes) was easily achieved. Characteristically sharp boundaries were always obtained, a proof that in isotachophoresis, these cells do behave analogously to proteins or other ionic constituents. However, fractionation of mixtures of sheep and rabbit cells or human and rabbit cells (i.e., separation into two clearly defined zones) was not achieved. Again, the customary assortment of spacers was used, including ampholines and amino acids. The addition of ampholines caused spreading of boundaries, proving that at least some ampholin fractions possessed mobilities comparable to the two cell populations. Lack of visible separation can be interpreted in terms of an interaction between the red blood cells and ampholine, resulting in randomization of cell mobilities. The situation was radically different with frozen mixtures of fresh cells. These cells readily separated in isotachophoresis even without the addition of spacers. Because freezing always results in partial hemolysis of the cell population, this was interpreted as showing that hemolysis liberated a spacer of suitable mobility to act as a spacer. No attempts were made to characterize this spacer. Attempts to use the supernatant from frozen fresh cells to separate fixed cells were not productive. For the ASTP experiment, it was therefore proposed to fly one sample of fixed cells and one sample of frozen fresh cells.

Isotachophoresis column 4 was processed using rabbit and human formalin-fixed red blood cells. The concentration of these cells, when loaded, was the same as that for columns 1 and 5. The volume of live red blood cells loaded into the sample slide (number 8) was provided in the following manner. Human and rabbit cells were prepared at a 40-percent hematocrit each. Equal volumes of each concentration were loaded into the sample slide. The final concentration was $450 \times 10^6$ cells/0.098 ml. The isotachophoresis sample slides had a slightly larger volume (0.098 ml) than that of the electrophoresis slides (0.06 ml).

On the day of the ASTP flight experiment, two sample slides containing duplicate samples of the fresh frozen flight cells were removed from liquid nitrogen, thawed, examined for free hemoglobin, and submitted to isotachophoresis in the Rotofor, using aliquot portions of the flight buffer. Free hemoglobin was found to correspond to a hemolysis of 52 percent of the cells, considerably higher than the usual hemolysis of less than 20 percent that was routinely observed in previous experiments. The decreased recovery of cells did not affect the ASTP experiment because sufficient cells survived to form good bands, and, indeed, the cell mass was clearly visible in both ground-based and ASTP runs. One might have expected the formation of a third isotachophoresis band containing free hemoglobin. Unfortunately, an isotachophoresis run in the Rotofor, under conditions of amperage and duration that duplicated the conditions of the ASTP flight, did not result in the expected separation of the two cell populations and/or hemoglobin. The migration distance was also lower than expected; the sample migrated only 30 mm in 45 minutes. The boundaries were quite diffuse and did not present the usual sharp fronts of other isotachophoresis cell runs. At the time of this experiment, there was no explanation for the lack of sample resolution. The lack of characteristically sharp boundaries is frequently observed during early stages of isotachophoresis before the sample is fully resolved and a steady state is reached. Lack of boundary sharpness is particularly pronounced in the presence of large amounts of ampholines, which provide a continuous mobility spectrum spacer. Migration velocity is also usually reduced during the resolving period of a mixture containing large amounts of ampholines, and visible samples frequently appear to remain stationary, whereas, in fact, higher mobility ampholine fractions are advancing into their proper position along the column.

Thus, this ground-based control experiment had all the appearances of isotachophoresis of a sample in the presence of a great excess of ampholines. At this time, only limited importance was assigned to this ground-based control experiment. The limited amount of available control-frozen cells prevented the exploration of this cell

behavior. In view of the similarly reduced migration velocity of the cells in the ASTP experiment, this control experiment proved to be highly significant. Its implication will be discussed, correlating it with the increased hemolysis of the sample.

## Evaluation of the Photographic Record of the Flight Experiment

Isotachophoresis of the formalin-fixed red blood cells was run in column 4. Photographs taken at various times are shown in figure 19-20. The photographic record is not as good as desired because the film was underexposed and the visibility is particularly poor at the ends of the column. The photographs were taken at 3-minute intervals. The first frame showed the current off; the following frames, covering a timespan of 40 minutes, showed the current on; and the last three frames showed the current off again. Thus, because no record was made at precisely the time the switches were turned on and off, it is not certain whether the current was on for 40 minutes, for 46 minutes, or for some interim time period. The migration velocity appears to be 10 mm in 9 minutes, or very close to the predicted velocity of 1 mm/min. A different velocity is obtained, however, if the entire timespan of migration is considered. The last photograph (fig. 19-20(d)) was taken at a minimum of 40 minutes and a maximum of 43 minutes of migration; thus, the overall migration rate was 0.725 or 0.675 mm/min, depending on the exact time of current application.

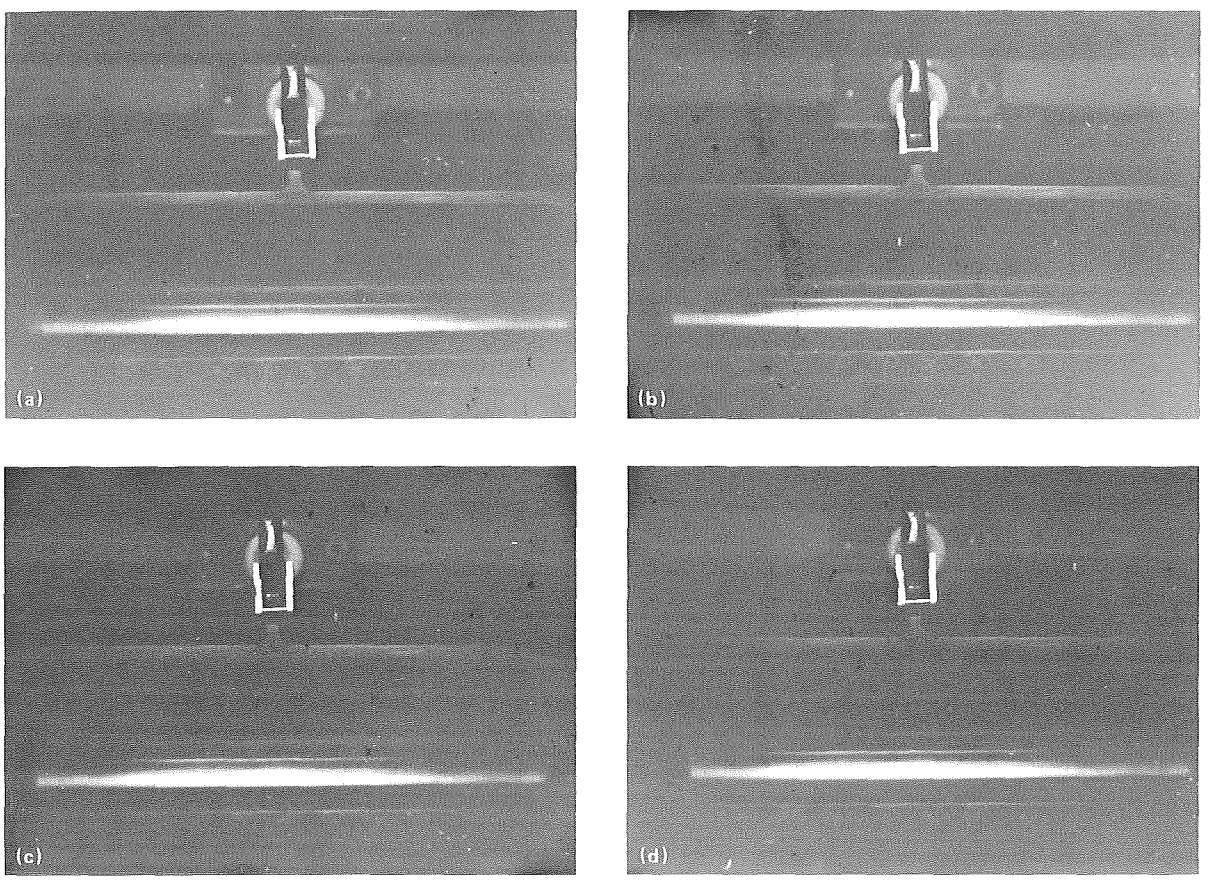



FIGURE 19-20.—Flight photographs of column 4. The sample is formalin-fixed rabbit and human red blood cells. (a) After 31 minutes of isotachophoresis. (b) After 34 minutes of isotachophoresis. (c) After 37 minutes of isotachophoresis. (d) After 40 minutes of isotachophoresis.

This value represents a substantially lower migration rate than expected.

The first visible boundary (fig. 19-20(a)) appears slightly fuzzy; but the last boundary, four frames later, is sharp and flat, as expected in isotachophoresis. The initial voltage was 113 V, and the final voltage was 133 V. This increase is characteristic of isotachophoresis. On the basis of the ground-based experiment, the predicted initial voltage would have been 106 V; thus, in this regard, there is excellent agreement.

The isotachophoresis of fresh-frozen cells occurred in column 8 (fig. 19-21). The quality of the photographs is somewhat better than for the first isotachophoresis run, and the column is visible past the 15-mm mark. In the case of column 8, the experiment was performed twice by the crewmembers; the first portion of the run showed that no current was applied and, consequently, no migration occurred. For unknown reasons, the run was repeated and the remaining 39-minute timespan showed the current to be on. This second run was initiated, however, at least 70 minutes after insertion of the sample specimen into the column. Thus, there is considerable uncertainty as to the position of the sample at the beginning of the run.

An overall migration rate of 1 mm/min was predicted for both samples. The last four frames of the fixed cells confirm this migration rate, but the overall migration rate appears to be substantially lower than expected (presumably only 0.675 mm/min). For the fresh cells, the migration rate was still lower, being in the range of 0.55 to 0.64

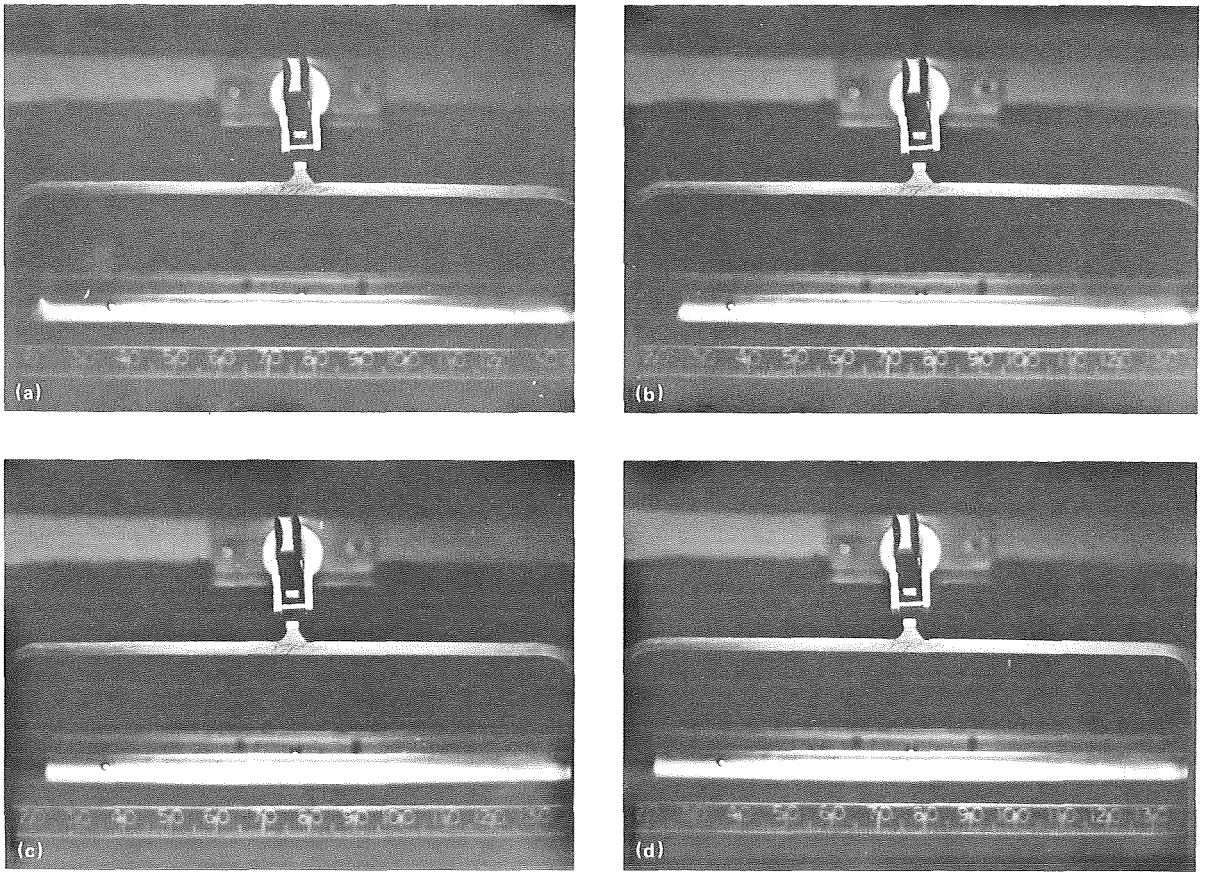



FIGURE 19-21.—Flight photographs of column 8. The sample is fresh frozen rabbit and human red blood cells. (a) After 30 minutes of isotachophoresis. (b) After 33 minutes of isotachophoresis. (c) After 36 minutes of isotachophoresis. (d) After 39 minutes of isotachophoresis.

mm/min, depending on the mode of calculation.

Interpretation of the mobility differences is difficult because there are several possible contributing factors: (1) there is an inherent uncertainty concerning overall duration of each run because the exact time of power application was not recorded; (2) for column 8, there appear to be unexplained periods of power failure; and (3) the position of the sample at the beginning of the fresh cell run is uncertain, as previously mentioned, due to the insertion of the sample at least 70 minutes before the onset of migration. Thus, it is not certain whether, during either of the isotachophoresis experiments, the photographs taken at 3-minute intervals represent a true record of run times. It is conceivable that power may have been flickering, but this was not observed during ground-based runs. Although measurements of advancing fronts in the fixed cell show constant increments, this is not the case with the fresh cell run.

One must assume, therefore, that none of the mentioned factors contributed to the observed reduction in migration velocity. That is, the same reduction in migration of the fresh cells in the ground-based control experiment was obtained when performed in the Rotofor using a duplicate of the flight sample. It will be remembered that this sample had an unusually high hemolysis. The reason for this high hemolysis is inexplicable. During the month before submission of flight samples, the cell preparation and freezing procedures were conducted numerous times, with innumerable individual samples, and uniform results were obtained. The separation of the two cell populations in frozen fresh cell isotachophoresis is predicated, however, on the liberation of an unidentified spacer. It is possible that the freezing procedure liberates not only a colorless spacer with intermediate mobility between the two populations, but also a spacer with a mobility higher than that of the human cells. Thus, a leading band of colorless spacer may be present. Increased hemolysis may account for greater quantity of either spacer, and thus result in slower initial migration rate, while the components of the mixture are resolved. As previously mentioned, this occurs whenever an excess of ampholines is used. Thus, the reduced rate of fresh cell migration may have been caused by excessive hemolysis, the sample not having reached a steady state in the time allotted.

A similar explanation may account for the discrepancy in the migration rate of fixed cells. That is, colorless fast ions may have been in the sample, thus retarding initially the migration rate of these cells. The sample, however, achieved a steady state of migration by the time the photographic record was obtained, as judged by the cell migration velocity.

In summary, the two isotachophoresis experiments did not run for a sufficient time to permit visualization of rear or intercompartmental boundaries. Nevertheless, the experiment shows the great advantage of isotachophoresis in the sharpness of the frontal boundary and in the concentration of the migrating zones. Thus, the main potential advantage of isotachophoresis is the quality of sample it may fractionate. With respect to cells, however, one must specify that the future of isotachophoresis depends on finding proper spacers.

## SUMMARY

The basic objectives of the Electrophoresis Technology Experiment were to conduct engineering and operational tests of a space-rated static electrophoresis separation apparatus and to further current research efforts through separation of similar cellular species. In addition, special effort was made to improve existing concepts in several areas. A method was needed that would allow for the precise application of the sample without affecting the subsequent electrophoresis. This improvement was accomplished by inserting the sample as a frozen disk. Attempts were made to separate and return viable lymphocytes and kidney cells. This goal was accomplished with the kidney cells but not with the lymphocytes. A technique was needed to demonstrate viable candidate preservation after separation. This preservation was accomplished for the first time in space by using a thermoelectric module to freeze the sample at a prescribed rate after separation was completed. The experiment allowed, therefore, the testing and evaluation of many important aspects

related to space-flight electrophoresis. In almost all cases, the concepts developed were proven operationally or shown to offer significant improvements over past efforts. Of particular interest was the low zeta potential coating. The minimization of electro-osmosis in this experiment provided a breakthrough sought since before the Apollo 14 separation attempts.

In summary, this experiment provided a significant step forward in the development of a biological processing facility in space. All areas of concern — i.e., sample preservation, sample collection and return, biocompatible systems, and sterile operating environment — were considered in this experiment. The accomplishments were significant and encouraging. The performance of electrophoretic separations in space without sedimentation and heat convective-mixing problems was demonstrated. The isotachophoresis of large living biological particles can be achieved. Scientists limited by Earth gravity problems can now use space-flight operations in the processing of particles that are difficult to separate or purify on Earth.

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